Immunology Letters, 40 (1994) 287–289
0165 – 2478 / 94 / $ 7.00

IMLET 02151

Age-associated changes in the B-cell repertoire: effect of age on RAG-1 gene expression in murine bone marrow

Arie Ben-Yehuda, Paul Szabo and Marc E. Weksler*

Division of Geriatrics and Gerontology, Cornell University Medical College, New York, N.Y. 10021, USA

(Accepted 29 January 1994)

1. Summary

The available repertoire of B cells that develop early or late in life reflects the restricted recombination of 5′ immunoglobulin heavy chain variable region genes. In addition, autoantibody reactivity is overrepresented in the preferentially recombined 3′ Vh gene families, resulting in over representation of autoreactive antibody specificities. The preferential utilization of 3′ Vh gene families is associated with, and perhaps caused by, the reduced expression of the RAG-1 gene. One can only speculate what effect the induction of RAG-1 activity in the bone marrow of old mice would have on their antibody repertoire.

2. Introduction

The antibody repertoire changes significantly with age. We have examined the age-associated shifts in antibody repertoire from four points of view: (i) antibody specificity, (ii) antibody heterogeneity, (iii) activity of genes that control immunoglobulin rearrangement (RAG-1 and RAG-2) and (iv) use of immunoglobulin variable region genes. The specificity of antibody produced during an immune response changes with age. Thus, there is an age-dependent decrease in antibody response to most foreign antigens accompanied by an increased non-specific autoantibody response as well as by the increased concentration of a regulatory autoantibody – auto-anti-idiotypic antibody [1,2]. The frequency of most serum autoantibodies increases with age. In the oldest subjects the frequency of some autoantibodies may decline, perhaps as the result of the shortened survival of persons with autoantibodies [3].

Immune senescence is also characterized by a decreased heterogeneity of the antibody response to antigen with respect to isotype and to binding affinity for antigen [4]. With increasing age, the antibody response becomes progressively more dominated by IgM and low affinity antibody as immunoglobulin class switching and the production of high affinity antibodies by somatic mutation decrease. It is reasonable to suggest that age-associated thymic involution and impaired peripheral T-cell function, known to influence both class switching and the production of antibodies with high affinity for antigen, may contribute to this aspect of immune senescence. The loss of the heterogeneity in the antibody response with age reflects a loss of preimmune clonal diversity among the available B-cell repertoire, as well as impaired somatic mutation, the mechanism that normally leads to high affinity antibody producing cells under the influence of antigen. The decline of B cells secreting high affinity, IgG antibodies specific for many foreign antigens results in 'holes' in the antibody repertoire and may contribute to the increased susceptibility of the elderly to infectious disease and their impaired capacity to develop protective immunity following immunization [5]. Another indication of the decreased heterogeneity of the antibody response with age is the increased frequency of benign monoclonal immunoglobulins in humans and experimental animals with age [6].

There are two major forces that shape the immune repertoire: (i) the diversity of the genetically available B-cell repertoire and (ii) the selective forces which act upon the available repertoire to create the actual B-

Key words: Vh gene utilization; RAG-1; B cell repertoire; Autoreactive; Age

*Corresponding author: Dr. M.E. Weksler, Department of Medicine, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, USA.

SSDI 0165-2478(94)00067-2

cell repertoire. The diversity of the available B-cell repertoire depends first of all on the combinatorial possibilities offered by the assortment of distinct genetic elements that contribute to the structure of the antibody [7]. In brief, this is accomplished by the joining of variable, joining, constant, and in the case of immunoglobulin heavy chains of diversity, gene segments. The variable segments are flanked by heptamer, nonamer recombination signal sequences which are the targets of the products of two closely linked recombination activation genes, RAG-1 and RAG-2 [8,9]. Both of these mRNAs are expressed at a high level in the thymus gland and bone marrow, the sites where diversification of the pre-immune lymphocyte repertoire occurs.

RAG gene expression is almost exclusively limited to pre-B or pre-T lymphocytes. Inactivation of either RAG-1 or RAG-2 genes in mice prevents productive immunoglobulin or T cell receptor gene recombination, a failure to produce either B or T cells with receptors for antigen and such mice have a severe combined immunodeficiency [10,11]. Thus, RAG-1 gene expression can be used as an index for the process of diversification of the B cell repertoire. In addition to the diversification of the pre-immune B cell repertoire, the expressed antibody repertoire is further shaped by junctional diversity and somatic mutation following exposure to antigen [reviewed in 12].

3. Vh gene utilization in old mice

Our studies compared Vh immunoglobulin gene use in 2-3 and 16-18 month old female C57BL/6 mice. The older mice showed a pattern suggesting preferential use of the 3′ proximal immunoglobulin Vh gene families 7183 and Q52 compared to the 5′ Vh gene family, J558. It is of note that these D proximal Vh immunoglobulin gene families, 7183 and Q52, were reported to encode autoantibodies more frequently than other Vh immunoglobulin genes [13]. This may explain the fact that old mice generate more autoreactive antibodies during an immune response than young mice. These same Vh gene families are preferentially utilized by neonatal mice and generate highly connected, cross-reacting antibodies many of which are natural auto-anti-idiotypic antibodies [14]. Thus, early and late in life, D proximal Vh gene families are preferentially recombined giving rise to a disproportionately large frequency of autoantibodies including auto-anti-idiotypic antibodies. Preferential utilization of 3′ immunoglobulin Vh gene families has also been reported in nude mice [15] and in mice maintained in an antigen-deprived environment [16].

4. Effect of age on RAG-1 activity in bone marrow

The association between age, thymic function or antigen exposure and preferred recombination of 3′ Vh immunoglobulin gene utilization raises the possibility that these states lack a biological activity necessary for random Vh immunoglobulin gene recombination. Alteration in the structure of the DNA coding for immunoglobulin and/or in the recombination complex might result in D proximal Vh immunoglobulin gene preference. The RT-PCR reaction was used to measure the level of bone marrow RAG-1 mRNA as a surrogate for a direct assay of the recombinatorial activity in bone marrow pre-B cells in old, nude and antigen deprived mice.

A necessary first step in such an analysis is the demonstration that all RAG-1 activity in bone marrow cell preparations is derived from B-cell precursors [15]. Once this was demonstrated, the effect of age on bone marrow RAG-1 activity was quantitated. In normal mice, the activity of RAG-1 in the bone marrow increases from birth to attain maximal activity at 2 months of age. Maximal activity is maintained between 2 and 5 months of age. Thereafter, RAG-1 activity decreases so that by 10 months of age bone marrow RAG-1 activity is only 10% of its maximal level. As nude and antigen-deprived mice also show preferential D proximal Vh immunoglobulin gene utilization, bone marrow RAG-1 activity was determined. Little or no RAG-1 activity was detectable in nude or antigen-deprived mice. Thus, in these three murine models, the preferential 3′ Vh immunoglobulin utilization is associated with a greatly reduced expression of the RAG-1 gene in bone marrow pre-B cells. Thus, the capacity of animals to recombine diverse Vh immunoglobulin gene segments appears to be associated with bone marrow RAG-1 activity.

It has been previously shown that preferential D proximal Vh immunoglobulin gene utilization in nude mice is eliminated following the injection of T cells [17]. For this reason, bone marrow RAG-1 activity was measured in nude mice after the injection of T cells. Injection of T cells not only increased bone marrow RAG-1 to normal levels but these animals gained the capacity to recombine the full diversity of Vh immunoglobulin gene families.

5. Age-associated shifts in antibody repertoire reflects limited diversity of Vh immunoglobulin gene recombination

The heterogeneity of the antibody response is built upon the potential diversity of recombination among the genetic elements that code for the heavy and light chain. For this reason, the selection of the potential repertoire from D proximal Vh gene families in old animals leads to the overexpression of autoantibody specificities encoded by these Vh gene families. Life-long exposure to self antigens combined with the loss of T-cell regulation of autoreactive B-cell expression leads to a breakdown of B-cell tolerance and to the expansion of auto-reactive B-cell clones. Furthermore, with age there is an increased activity of one subset of autoreactive antibodies, auto-anti-idiotypic antibodies [2]. We have shown that these antibodies have the capacity to inhibit secretion of antibodies to nominal antigens, thus further skewing the balance between antigen-specific antibodies and antigen-non-specific antibodies which develop during the immune response.

Acknowledgements

The studies described were supported in part by NIH grants AG00541 and AG08707. A. Ben-Yehuda is the recipient of NIH Fogarty International Fellowship 1F05 TWO4552.

References

[1] Bovbjerg, D.H., Kim, Y.T., Schwab, R., Schmitt, K., DeBlasio, T. and Weksler, M.E. (1991) Cell. Immunol. 135, 519.

[2] Goidl, E., Thorbecke, G.J., Weksler, M.E. and Siskind, G.W. (1980) Proc. Natl. Acad. Sci. USA 77, 6788.

[3] Mariotti, S., Sansoni, P., Barbesino, G., Monti, D., Cossarizza, A., Giacomelli, T., Passeri, G., Fagiolo, U., Pinchera, A. and Franceschi, C. (1992) Lancet 339, 1506.

[4] Goidl, E., Innes, J.B. and Weksler, M.E. (1986) J. Exp. Med. 144, 1037.

[5] Wali, A., Cherniack, E.P., Ehleiter, D., Doria, G., Gross, P., Russo, C. and Weksler, M.E. (1992) Aging: Immunol. Infect. Dis. 3, 185.

[6] Radl, J. (1990) Immun. Today 11, 234.

[7] Tonegawa, S. (1983) Nature 302, 57.

[8] Schatz, D.G., Oettinger, M.A. and Baltimore, D. (1989) Cell 59, 1035.

[9] Oettinger, M.A., Schatz, D.G., Gorsha, C. and Baltimore, D. (1990) Science 248, 1517.

[10] Mombaerts, F., Iacomini, J., Johnsjon, R.S., Herrup, K., Tonegawa, S. and Papaioannou, V.E. (1992) Cell 68, 869.

[11] Shinkai, Y., Rathbun, G., Lam, K.P., Oltz, E.M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A.M. and Alt, F.W. (1992) Cell 68, 855.

[12] Blackwell, T.K. and Alt, F.W. (1988) in: Molecular Immunology (B.D. Harnes and D.M. Glover, Eds.) IRL Press. pp. 1–60.

[13] Bona, C.A. (1988) Ann. Rev. Immunol. 6, 327.

[14] Holmberg, D. (1987) Eur. J. Immunol. 17, 399.

[15] Grandien, A., Coutinho, A., Andersson, J. and Freitas, A.A. (1991) Int. Immunol. 3, 67.

[16] Freitas, A.A., Viale, A.C., Sundblad, A., Heusser, C. and Coutinho, A. (1991) Proc. Natl. Acad. Sci. USA 88, 5640.

[17] Freitas, A.A., Lembezat, M.P. and Rocha, B. (1989) Int. Immunol. 1, 398.